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(54) Title: PRO-PROTEIN CONVERTING ENZYME

(57) Abstract

A cDNA clone encoding the human prohormone convertase PC5 was isolated from human adrenal gland messenger RNA. The deduced protein sequene would encode a 915 amino acid prepro PC5 which shares a very high degree of homology with the previously cloned rat and mouse homologues. PC5 mRNA is detected in multiple human tissues, including the brain, adrenal and thyroid glands, heart, placenta, lung and testes. PC5 mRNA was undetectable in the liver and is present at lower levels in skeletal muscle, kidney, pancreas, small intestine and stomach. Co-transfection of human PC5 and human prorenin expression vectors in cultured GH_4Cl cells leads to secretion of active renin. The activation of human prorenin by PC5 is dependent on a pair of basic amino acids at positions 42 and 43 of the prorenin prosegment and occurs only in cells containing dense core secretory granules. Human PC5 was co-localized with renin by immunohistochemistry in the zona glomerulosa of the adrenal gland suggesting that it could participate in the activation of a local renin-angiotensin system in the human adrenal cortex. PC5 is overexpressed in atherosclerotic coronary blood vessels. Silencing PC5 expression with a specific antisense oligonucleotide efficiently inhibited the proliferation of smooth muscle cells in culture. Furthermore, the antisense inhibited carotid stenosis in a carotoid injury model. These results indicate that silencing PC5 applies to the prevention of restenosis. PCs could be targets of choice for treating any proliferative diseases involving their action on a given growth factor. Finally, the antisense oligonucleotide PC5 is to be used for silencing the activity of this enzyme towards HIV gp160, since both coexist in $CD4^+$ T lymphocytes and the viral glycoprotein is cleavable by PC5.

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TITLE:

PRO-PROTEIN CONVERTING ENZYME

FIELD OF THE INVENTION:

This invention relates to protein processing enzymes or pro-hormone convertases (PCs), specifically to PC5, more specifically to the human PC5.

BACKGROUND OF THE INVENTION:

Pro-hormone convertases (PCs) belong to a family of enzymes responsible for the maturation of proteic precursors into active proteins or enzymes. Up to now, many human enzymes of that family have been identified, namely furin, PC1, PC2, PC4 and PC7. Each enzyme has a tissular distribution which may be restricted (for example, PC4 is restricted to male germ cells) or ubiquitous (furin is such an example). Although all these enzymes share the properties of cleaving precursor proteins at basic or dibasic residues, they nevertheless have differing cleavage specificities. The action of a specific pro-hormone convertase is therefore governed by the cleavage sequence of a given protein substrate, and/or by the location of that enzyme in a tissue expressing or responding to a given proteic substrate growth factor or hormone.

Renin is an aspartyl protease which makes an important contribution to cardiovascular physiology and pathophysiology through its key role in the synthesis of the vasoactive octapeptide angiotensin II (AII). While the kidney is the primary source of circulating active renin, several additional tissues, including the pituitary and adrenal glands, placenta, uterus, ovary, testes, heart, vasculature and brain express the renin gene (reviewed in [1-4]). The presence of additional components of the RAS (renin-angiotensin system) in these tissues, including angiotensin converting enzyme (ACE) and angiotensin II receptors, has led to the proposal that certain tissues might contain a locally active tissue renin-angiotensin system (tRAS) although the actual function of the various tRAS is still largely a matter of conjecture.

Renin is first synthesized as an enzymatically inactive precursor, prorenin, which is converted to active renin by the proteolytic removal of a 43 amino acid amino-terminal prosegment. The activity of the RAS within any given tissue would, therefore, be dependent on the existence of proteolytic enzymes capable of converting prorenin to active renin and on the expression of such prorenin processing enzymes (PPEs) in the same cells that express prorenin. The identity of the enzyme(s) responsible for the proteolytic activating human prorenin *in vivo* is still uncertain. Furthermore, it is possible that multiple PPEs exist in humans and these may differ among renin-producing tissues. Biochemical and microscopic studies of renin in the kidney suggest that candidate PPEs should be selective for cleavage of human prorenin at Lys^{42}, Arg^{43} of the prosegment[5] and would be active in secretory granules of the juxtaglomerular (JG) cells.[6] The lysosomal enzyme cathepsin B has been co-localized with human renin/prorenin in the secretory granules of JG cells and human pituitary lactotrophs[7,8] and has been shown to cleave human prorenin *in vitro* with a high affinity and selectivity for the proper cleavage site.[9] The prohormone convertase PC1 has also been shown to cleave human prorenin with the correct site-and organelle specificity in transfected cells[10] and to co-localize with renin in the adrenal medulla and derived tumors[11], but not in JG cells.[12]

In an effort to identify novel PPEs, we recently determined the distribution of processing enzymes in an established renin-expressing tissue culture cell line derived from an oncogene-induced mouse tumor (As4.1 cells[13]). One such enzyme, the mouse prohormone convertase PC5, was found. Mouse PC5 is capable of partially cleaving human prorenin.

Miranda et al. (38) describe the cDNA and protein sequences for a human PC6 enzyme obtained by PCR from $CD4^+$ T lymphocytes. PC5 and PC6 are different names given to what appears to be the same enzyme. However, the sequences of Miranda et al. comprise a plurality of substitutions when compared to the present PC5 sequences. Moreover, the size of messenger RNA encoding PC6 and PC5 are similar but not identical. Since the present PC5 sequences were obtained from

human adrenals, both enzymes may be isoforms, differentially expressed in tissues and they may have different activities.

Prorenin and HIV gp160 are most probably not the only proteic precursors to be recognized and cleaved by PC5. Many growth factors responsible for cell proliferation are cleaved by one or more PCs: they include platelet-derived-growth factors A and B (PDGF's), epidermal-growth-factor (EGF), insulin-like growth factors I and II (IGF's), transforming growth factors α and β (TGF's). Each of these named growth factors has the typical cleavage site motif K/R-$(X)_n$-R↓ (where n=0,2,4,6). Full biological potency is conferred to these growth factors only after cleavage at these sites, by one or more of the PC enzyme family. There is therefore a possibility that manipulating the expression of the PCs would affect cell proliferation via deficient growth factor activation.

Out of the >450,000 patients/year in the U.S. and Canada who undergo percutaneous transluminal coronary angioplasty (PTCA), 30-50% of them will restenose their coronaries within 3-6 months. This flare-up of endothelial and smooth-muscle cells proliferation is due to the activation of numerous regulatory growth factors. Therefore, knowing which enzyme(s) is (are) responsible for this activation, and manipulating the level of expression of this or theses enzyme(s) would be particularly useful to prevent restenosis.

STATEMENT OF THE INVENTION:

The present invention relates to the human PC5 (hPC5). We demonstrate that hPC5 isolated form human adrenals proteolytically activates human prorenin with the expected site- and organelle- specificity and that it is co-expressed with prorenin in the zona glomerulosa of the adrenal cortex. Therefore, PC5 is a prorenin-processing enzyme (PPE). Silencing the expression of PC5 would find a specific application in inhibiting the production of renin, and a method of inhibiting the production of renin is an object of the invention. Since the production of renin is one of targets of the RAS involved in hypertension. Furthermore, we demonstrate that hPC5 is overexpressed in atherosclerotic

coronary arteries. Antisense oligonucleotides have been designed, amongst which one has been shown to successfully silence the expression of hPC5 in smooth muscle cells in culture. This antisense inhibited carotid stenosis in a *in vivo* rabbit carotid injury model. These results indicate that a method of silencing the expression of PC5 would find a specific application in preventing restenosis.

PC5 is known to be expressed in $CD4^+$ T cells, along with furin and PC7. The three enzymes are capable of converting HIV gp160 into its fusiogenic form. Therefore, antisense constructs, particularly the oligonucleotide that successfully inhibited restenosis, will find a use in inhibiting expression of the activity of PC5 towards HIV gp160.

The complete amino acid and nucleotide sequence of hPC5 is described hereinbelow and are another object of this invention. Recombinant vectors and hosts comprising as a new insert, whole or part of hPC5, are also an object of the invention.

Oligopeptides derived from the proteic sequence of hPC5 are also an object of the invention.

Antibodies directed against the whole protein hPC5 or a part thereof are also an object of the invention.

Diagnostic methods and kits comprising oligonucleotides or antibodies binding PC5 nucleic acids or protein or peptides are also an object of the invention.

This invention will be described hereinbelow by way of specific embodiments, examples and figures which purpose is to illustrate the contemplated aspects of the invention, and not to limit the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic diagram of the isolated cDNAs encoding hPC5. Restriction enzyme sites used in sub-cloning are denoted. Solid lines represent clones isolated from a phage library. Hatched lines denote the portion of the cDNA isolated by RT-PCR of human adrenal mRNA. The double line represents the portion of the mouse PC5 cDNA (corresponding

to the amino terminus of the signal peptide) which was used to complete the cDNA for expression.

Figure 2: Nucleotide and derived protein sequence of hPC5 (SEQ ID NOS: 1 and 2, respectively). Proposed signal peptide (solid arrow) and prosegment (open arrow) cleavage sites are denoted based on data from mouse PC5.[15] The underlined sequence represents the portion of the signal peptide from mouse PC5 which was used in the expression vector construction.

Figure 3: Distribution on PC5 RNA in various human tissues. Each lane contains 2 μg poly-A RNA. Filters were hybridized with a radiolabeled probe for hPC5 as described in Materials and Methods. Shown at left is the migration of single strand size standards in kilobases (Kb). Note that the absolute signal cannot be compared between the two filters as they were of different ages and hybridized at different times.

Figure 4: hPC5 cleaves human prorenin with site and cell specificity. **Panel A:** GH_4C_1 cell were co-transfected with expression vectors for the indicated proteins. Supernatants were collected 30 hrs. after transfection and assayed for % active renin [(active renin/total renin) X 100]. Bars represent the mean ± S.E.M of 9 independent transfections. * = P< 0.0001 as compared to proren + pUC, as determined by the Mann-Whitney non-parametric test. **Panel B:** Resulting secretion of active renin after co-transfection of CHO cells with an expression vector for prorenin and either a control plasmid (pUC) or hPC5. Bars represent the mean ± S.E.M of 3 independent transfections.

Figure 5: Active renin generation in secretory granules of co-transfected GH_4C1 cells. Parallel wells of GH_4C1 cells co-transfected with expression vectors for human prorenin and human PC5 were incubated for 20 min. in medium containing either 50mmol/L NaCl (control) or 50mmol/L KCl (a de-polarizing agent which causes the acute release of secretory granules). Percent active renin was calculated as described in the legend to Figure 4A. Bars represent the mean ± SEM of 3 independent transfections. * = P< 0.005 using Student's t-test.

Figure 6: Immunodetection of hPC5 and renin/prorenin in renal cortex, human placental cotyledon and adrenal gland. Positively stained areas are denoted by solid arrows. Sections in adrenal cortex are separated by 5mM to show co-localization in the cells of the zona glomerulosa (g) and absence of staining in the capsule (c) and zona fasciculata (f). Original magnification 25X (kidney and placenta) and 80X (adrenal gland).

Figure 7 : *In vivo* hybridization analysis of PC5 mRNA in human coronary blood vessels in atherosclerosis. The lower panel show a vessel where a severe lesion was observed. PC5 mRNA was abundantly expressed in this vessel, in the smooth muscle cells in the neointimal formation (see arrows). In comparison, another vessel which is free of any lesion, did not express PC5 mRNA (shown in the upper panel).

Figure 8: Western blot analysis of PC5 protein in rabbit smooth muscle cells treated with either antisense, sense or mismatch PC5 oligonucleotides. The specific PC5 band is identified (see arrow) by comparison with proteins extracted from rat supraoptic nucleus (SON) and SK-N-MCIXC cells (human neuroepithelioma). In the sample extracted from antisense PC5 treatment, we observe a dramatic decrease in the level of PC5 signal (approximately 2-3 fold decrease) in comparison to the control sense or mismatch PC5 oligos. This indicates that the antisense treatment reduced significantly the protein levels of PC5 in rabbit smooth muscle cells.

Figure 9 : Rabbit *in vivo* test of the PC5 antisense ODN as compared to the control sense and random ODNs. It shows a decreased stenosis due to the presence of a PC5 antisense when compared to the sense and random controls.

Figure 10 : Proprotein convertase immunoreactivity in human atherectomy specimens. It shows the presence of the enzymes of the pro-hormone convertase family which are present in the specimens.

Figure 11: Illustrates differences between cDNA sequences of PC6 (Miranda et al. SEQ ID No. 4) and PC5 (present invention).

Figure 12: Illustrates differences between protein sequences of PC6 (Miranda et al. SEQ ID No. 5) and PC5 (present invention).

DESCRIPTION OF THE INVENTION

Materials and Methods

cDNA library construction and screening: A cDNA library derived from total human adrenal RNA was constructed by Stratagene (La Jolla, CA) in the phage vector Uni-Zap XR. Six hundred thousand phage plaques were screened initially using radioactive probes and standard methodologies.[14] The initial hybridization probe was a 320 base pair DNA fragment derived from reverse-transcriptase PCR of human brain RNA using information derived from an unidentified human cDNA sequence tag in Genbank (Accession # M85522) with a high degree of similarity to the previously cloned mouse PC5.[15] Fragment labeling was carried out using ^{32}P dCTP and a random primer labeling kit (Boehringer-Mannheim Canada, Laval, Quebec, Canada) according to manufacturer's instructions. One positive hybridizing phage (hPC5A) was identified. Its insert was sequenced in its entirety using the dideoxy-chain termination method and found to code for an 1150 base pair cDNA with a high degree of sequence similarity to mouse PC5 (data not shown). A 1070 base pair fragment (excluding the poly A tail) was excised from hPC5A, labeled and used to re-screen an additional 600,000 phage from the cDNA library. A second phage clone (hPC5B) was isolated and found to contain an 1807 base pair cDNA insert overlapping hPC5A and extending toward the 5' end of the cDNA (Figure 1).

Reverse-transcriptase PCR: One microgram of poly A+ RNA from total human adrenal (Clontech Laboratories, Palo Alto, CA) was subjected to reverse transcriptase polymerase chain reaction (RT-PCR) using a published procedure[16] and the following oligonucleotides:

Forward oligonucleotide; derived from a region corresponding to the signal peptide of mouse PC5.[15] An artificial HindIII restriction enzyme cleavage site added to the 5'-end of the amplified

fragment for the purpose of cloning is underlined:

5'-CCAAGCTTGGCTGCTGTGCGTGCTGGC-3'

Reverse oligonucleotide; derived from the 5'-end of the phage hPC5B. An internal BglII restriction enzyme site is underlined: 5'-CTGCCTCAGATCTGTAGTG-3'

The entire RT-PCR reaction was repeated 4 times and 4 independently derived clones of the amplified fragment were sequenced and the sequences were compared. The sequence submitted to Genbank (Accession #U49114) represents the consensus sequence, defined as any nucleotide appearing in 3/4 clones.

Northern blot analysis: Tissue distribution of PC5 mRNA was determined by hybridizing commercially purchased nitrocellulose filters containing aliquots (2μg) of Poly-A RNA from various human tissues (Clontech Laboratories, Palo Alto, CA). The probe used was a complementary RNA derived from the full length hPC5 cDNA. Probe labeling and hybridization were carried out as previously described.[17]

Expression vector construction: A cDNA fragment from the KpnI site (Figure 1) to just past the stop codon was excised from the phage hPC5B and combined with a KpnI to HindIII (see above) fragment derived from portions of two independent RT-PCR clones (so as to eliminate errors arising from the Taq polymerase). A region corresponding to the first 16 amino acids of the signal peptide derived from mPC5 was attached to the 5'-end by overlap-extension PCR.[18] Thus, the entire cDNA, encoding amino acids 1-16 derived from the mPC5 signal peptide and the remainder from hPC5, was subcloned into the expression vector RSV-globin[19] which places the cDNA under the control of the RSV promoter and provides a 3' intron and polyadenylation signal from the rabbit beta globin gene. The entire subcloned fragment was subsequently verified by DNA sequencing.

Cell culture and transfection: GH_4C1 cells were plated in 6-well culture dishes at a density of $5X10^5$ cells per well. Twenty four hours later, medium was changed and the cells were transfected by the DEAE-dextran method using a commercial kit (CellPhect Transfection kit, Pharmacia Biotech, Baie D'urfe, Quebec, Canada) according to manufacturer's instructions. Each well received 0.18 μg of

either the hPC5 expression vector or a neutral plasmid vector (pUC18) and 0.18 μg of an expression vector for human prorenin (pRHR1100) or its equivalents in which amino acids 42 or 43 of the prorenin prosegment were mutated to alanine (K/A -2 and R/A-1, respectively[20]). Supernatants were collected 30 hrs. after transfection and assayed for prorenin and renin content as previously described.[20]

To verify that conversion of the prorenin occurred in the secretory granules, GH_4C1 transfected with the human prorenin and hPC5 expression vectors were stimulated to release secretory granules by depolarization using a previously published technique.[21] Forty hrs. after co-transfection, the culture medium in parallel wells of transfected cells was replaced with pre-warmed medium supplemented to a final concentration of 50 mmol/L with either NaCl (control) or KCl (secretagogue). The media were collected after 20 min. and assayed for renin/prorenin. A potassium-dependent increase in the percent active renin contained in cell supernatants was taken as an indication of active renin release from the secretory granules of the transfected cells. Results shown in Figure 5 represent the mean of three independent transfection experiments.

Immunolocalization of hPC5 in human tissues: Human tissue was obtained post-mortem (kidney and adrenal gland) or post-partum (placental cotyledon), fixed in Bouin's solution and embedded in paraffin. For immunolocalization, 5μm sections were mounted on gelatin-coated slides, deparaffinized and incubated with a 1:50 dilution of a polyclonal rabbit antiserum raised against a peptide corresponding to the N-terminal 16 amino acids of rat PC5 (PC5.MAP antibody) or a 1:200 dilution of a polyclonal rabbit antiserum against recombinant human prorenin. For kidney and placental specimens, immune complexes were revealed by incubation with protein A-colloidal gold (15 nm particles) synthesized from tetra-chloroauric acid (BDH) according to the method of Ghitescu and Bendayan.[22] Gold particles were enhanced for viewing in the light microscope by incubation with silver (IntenSE™M Silver Enhancement Kit, Amersham Life Science, Oakville, Ontario, Canada) and sections were

counter-stained with hematoxylin and methyl green. Immune complexes on human adrenal sections were detected with a 1:200 dilution of biotin-labeled donkey anti-rabbit IgG and a 1:300 dilution of streptavidin-horseradish peroxidase complex (Amersham Life Science, Oakville, Ontario, Canada) and were incubated with diaminobenzidine and hydrogen peroxide (Sigma Chemicals, St. Louis, MO) as chromogen. All positive staining patterns were subsequently verified for specificity by omission of the first antibody.

Results

The primary sequence of human PC5 is shown in Figure 2. We were unable to clone the extreme 5'-end of the cDNA either by the RACE protocol[16] or by using oligonucleotides based on the published sequence of mouse PC5[15,23], possibly due to a high G/C content of the cDNA in this region. However, based on the published cDNA sequences for rat and mouse PC5[15], we are confident that we have isolated all but the 5'-most portion of the cDNA corresponding to the first 12 amino acids of the signal peptide. By comparison with the published sequence of mouse PC5, we predict that the cDNA isolated would code for a preproPC5 of 915 amino acids, including a signal peptide and a prosegment of 32 and 84 amino acids, respectively. The deduced sequence of hPC5 is 88% identical to the previously published mouse PC5 cDNA and 96% identical to the mouse PC5 protein.

Northern analysis of poly A RNA from a variety of human tissues reveals a major band of approximately 6.6 Kb and a minor band at approximately 3.8 Kb (Figure 3). PC5 RNA is detected in the brain, heart, placenta, lung, thyroid gland and testes and at lower levels in the skeletal muscle, kidney and pancreas, small intestine and stomach. In the adrenal gland, PC5 is particularly enriched in the cortex (Figure 3).

Because PC5 RNA appears to be expressed in a number of tissues previously reported to contain active renin, we have tested the ability of hPC5 to cleave human prorenin in a cell co-transfection assay (Figure 4A). As has been previously reported[10], when cultured rat sommatotrophic GH_4C1 cells are co-transfected with an expression vector encoding human

prorenin and a neutral plasmid vector, only unprocessed prorenin is secreted into the culture supernatant. In contrast, if the human prorenin expression vector is co-transfected with an expression vector encoding human PC5, a portion of the expressed prorenin is secreted as active renin. Co-expression of human PC5 with prorenin mutated at either of the basic residues forming the native cleavage site (Lysine 42 or Arginine 43) prevents activation. These results suggest that human PC5 activates human prorenin by proteolytic cleavage at the site previously reported for activation of renin in humans.[5] While human PC5 cleaves human prorenin in GH_4C1 cells, there is no apparent increase in active renin secretion when co-transfections are carried out in Chinese Hamster Ovary (CHO) cells (Figure 4B). One obvious difference in the CHO cell line as compared to GH_4C1 cells is their lack of secretory granules, suggesting that either human PC5 or human prorenin or both require the secretory granule environment for this proteolytic step. This conclusion is supported by the acute increase in active renin detected in the supernatants of co-transfected GH_4C1 cells treated for 20 min. with potassium chloride (Figure 5), a de-polarizing agent which causes the release of secretory granules.[21]

Using a polyclonal antibody raised against a peptide derived from mouse PC5, we have studied the distribution of human PC5 in several human tissues (Figure 6). To date, we have been unable to detect staining for PC5 in the human kidney, although our sections stain positively for renin. In the placental cotyledon, PC5 is located in the syncitiotrophoblast layer of the chorionic villi while anti-renin antibody stains primarily the chorionic mesoderm. In the adrenal gland, the antibodies against both renin and PC5 show a preferential staining of zona glomerulosa cells in the adrenal cortex (g) with very little staining of the capsule (c) and zona fasciculata (f). No staining was evident with omission of the first antibody (data not shown). Thus, our immunohistochemical studies would suggest that, of the three tissues studied, it is likely that prorenin and PC5 are only

clearly co-localized in the zona glomerulosa of the human adrenal cortex.

Discussion

In the present study, we describe the cloning and expression of the human prohormone convertase PC5 and its activity as a human PPE. Co-transfection assays in cultured cells demonstrates that hPC5 activates human prorenin with the expected site-specificity and that this cleavage most likely takes place in dense core secretory granules. In addition, immunohistochemistry of human tissues shows co-localization of hPC5 with renin in the zona glomerulosa of the adrenal cortex.

Several lines of evidence suggest that the human adrenal gland contains a physiologically important local RAS: First, RNA encoding angiotensinogen and renin have been detected in preparations from the human adrenal zona glomerulosa, fasciculata and medulla[24,25], confirming that both renin and its substrate are synthesized within the human adrenal gland. Second, ACE inhibition or blockade of angiotensin receptors inhibits aldosterone release from human adrenal tissue explants[26], suggesting that the local RAS plays an active role in the regulation of aldosterone secretion from the adrenal gland. Third, tissue explants of human adrenal cortex and aldosterone-secreting adenomas secrete small quantities of active renin[24,26,27], suggesting that the adrenal cortex expresses a PPE capable of activating human prorenin. Our current results suggest that PC5 could be the PPE responsible for activation of renin in the human adrenal cortex as both renin and hPC5 are immuno-detectable in the zona glomerulosa. Additional circumstantial evidence supports this conclusion: First, centrifugal fractionation of adrenal cortical cells reveals that renin is contained in the "granular" fraction, of intermediate density between vesicles and lysosomes.[28] As our current study suggests that PC5 only cleaves human prorenin in cells containing secretory granules, renin would be in the appropriate intracellular compartment to be activated by PC5 in the adrenal cortex. Second, rats transgenic for mouse Ren-2 renin [TGR(mRen-2)27] display

fulminant hypertension[29] which correlates best with the expression of the mouse prorenin in the adrenal gland.[30-32] As previous studies have demonstrated that PC5 is capable of activating mouse Ren-2 prorenin, but not rat prorenin ([23]and data not shown) it is possible that the TGR(mRen-2)27 transgenic rat is a model for activation of a tissue RAS by the fortuitous juxtaposition of prorenin with an appropriate PPE in the adrenal cortex. These results also raise the possibility that the tissue-distribution of PPEs and their apparent selectivity in activating prorenin from different species could lead to differing functions of the tissue RAS between rodents and humans.

The principal source of circulating active renin in humans is the JG cells of the kidney. Although low levels of hPC5 RNA can be detected by Northern blot analysis in a sample of total kidney poly-A RNA (Fig. 3), we were unable to localize PC5 immunostaining in kidney sections (Fig. 6) raising the possibility that PC5 is expressed at low levels in diffuse cell types in the kidney. Thus, while these results do not formally rule out PC5 as a PPE in the kidney, our inability to detect it in JG cells makes it unlikely that it plays a major role in the production of renal renin. In contrast, relatively abundant amounts of PC5 mRNA and protein were detected in the placenta although evidence suggests that placental cells in culture[33] and *in vivo*[34] only secrete prorenin. However, immunostaining revealed that the cells producing PC5 and prorenin in the human placenta are distinct. It is also unlikely that PC5 would activate prorenin once the two proteins are secreted due to the apparent requirement of a granular environment for the cleavage of prorenin by hPC5 in transfected cells. Thus, in contrast to the case in the adrenal gland, it is unlikely that PC5 expressed in the human placenta would activate placental prorenin.

In the mouse, two forms of hPC5 have been predicted based on cloned cDNAs; the first would be analogous to the hPC5 cDNA described in this study and to that cloned from rat tissues[15,23] while the second, called PC6B, is extended at its 3′-end due to a differential RNA splicing event.[35] Although

the hPC5 cDNA we have cloned is only roughly 3 Kb in length, the major RNA band seen in human tissues is of approximately 6.6 Kb. The identity of the longer band hybridizing to the hPC5 probe is currently unknown. It should be noted that neither of the cDNA clones isolated from a screening of 1.2 million phage from the adrenal library was extended at its 3'-end (Figure 1), although the probes used in their isolation cover the region of homology with the mouse PC6B variant.[35] In mouse tissues, expression of the PC6B variant is restricted to few tissues[35] while the abundance of the 6.6Kb detected with the hPC5 probe is directly proportional to the abundance of the 3.8Kb band. Hybridization of RNA blots from rodent tissues using a PC5 probe also reveals RNA bands of 3.8, 6.5 and 7.5 Kb[15,35] and use of a PC5-specific probe reveals a band at 6.5 Kb. Thus, it is possible that additional PC5 RNA species exist in mammals that are extended at their 5'-ends. Alternatively, human tissues may be particularly enriched in a homologue to PC6B which was not picked up in our screenings. Recent data suggest that the alternate C-terminal tail present on PC6B may serve to retain the enzyme in the Golgi network, while the "short" form of mouse PC5 is targeted to dense core secretory granules (N.G. Seidah, unpublished). These data and the results of our co-transfection assays (Fig. 4) would suggest that the "short" form of hPC5 described here is the form which would be active in renin processing in secretory granules.

The PC5 enzymes isolated from humans and mice show a remarkably high degree of conservation at the nucleotide and protein sequence levels. This degree of similarity is higher than that seen for the other mammalian PC enzymes which seem to diverge in the C-terminal half of the enzyme.[36,37] This high degree of sequence conservation may reflect an essential function of PC5 (and the C-terminus of PC5) in mammals.

PC5 is linked to smooth muscle proliferation

To investigate which PC could be a potential target of smooth muscle cell proliferation, we tested if any of the PCs were affected in the process of restenosis, wherein such proliferation is observed. Changes in PC levels in the

process of restenosis is a distinct possibility since in previous studies using animal models or cell lines, we have shown that PC levels can be regulated or even be induced. We thus obtained human restenosed coronary tissues from patients. These tissues were screened for each of the PC mRNAs using *in vivo* hybridization histochemistry in order to obtain information within an anatomical context. Coronaries with partial or total occlusions demonstrated dramatically increased PC5 mRNA levels within smooth muscle tissues, whereas coronary tissue without occlusions were PC5 negative. These results indicate that PC5 is either strongly up-regulated or induced in the human coronary arteries during the active process of stenosis (fig. 7). To our knowledge this is the first indication that a specific PC is directly linked to smooth muscle proliferation.

These results suggested that if PC5 enzymatic activity could somehow be inhibited or the upregulation of PC5 mRNA could be prevented, this may attenuate or stop the process of restenosis. This could occur through the inhibition of the processing function of this enzyme on the numerous growth factors that are involved in the formation of the coronary lesion. If these growth factors are not processed they will remain biologically inactive. Our approach was to test the effectiveness of PC5 antisense inhibition on smooth muscle proliferation *in vitro*.

A specific antisense oligonucleotide (ODN) was shown to drastically inhibit smooth muscle proliferation using an *in vitro* model of rabbit smooth muscle in culture. Incubating rabbit smooth muscle cells with a PC5 antisense 17-mer oligonucleotide shown in Table 1 caused a dose-dependent inhibition smooth muscle proliferation with a maximal inhibitory effect of 81.6% + 1.6% at 10 mM (mean of three experiments done in quadruplicates). This inhibitory effect is highly significant (P=0.0001) as compared to controls which included either a sense or a mismatched oligonucleotide used at the same concentration (see Table 1). In addition we found that the expression of PC5 is decreased in the affected cells (fig. 8). When compared to other targets, such as *c-myc*, this approach was much more effective in inhibiting

smooth muscle proliferation, as the best effects of antisense *c-myc* resulted in 71.7 + 3.5% (means of three experiment done in quadruplicate) inhibition (mean of three experiment done in quadruplicates). These results are indicative of an *in vivo* effect since silencing PC5 would impede muscle cell proliferation and restenosis.

Cholesterol conjugation of oligonucleotides

Phosphorothioate antisense ODNs were synthesized on a DNA/RNA synthesizer following standard procedure (Applied Biosystems). Conjugation of oligomers with cholesterol was achieved with 3'-cholesterol-VN CPG (Clontech), a virtual nucleotide (VN) glass reagent that introduces a cholesterol label to the 3' terminus of an oligonucleotide via solid-phase synthesis. When ODN synthesis. When ODN synthesis was completed, oligomers were removed from the column with 30% NH_4OH (1 hour at room temperature), and then deprotected for 8 hours at 60°C. Oligos were purified and detritylated with oligonucleotide purification cartridges (Applied Biosystems), and then lyophilized with a centrifugal evaporator (Savant SpeedVac).

***In vivo* arterial ODN transfection**

New Zealand rabbits male or female (2 Kg) were intramuscularly sedated with xylazine (2 mg/Kg) and anesthetized with ketamine (100 mg/Kg) prior to surgical exposure of left carotid artery. Segments of 10 mm of carotids were transiently isolated by temporary ligatures and rinsed with 0.9% sodium chloride via a cannula until there was no more visible evidence of blood components. Carotid arteries were transfected with 80 µmol/L of antisense ODNs in a 1 cm portion either alone or conjugated to cholesterol for a period of 30 minutes. The volume infused was 100 µl, and no visible loss of volume was noted throughout the incubation period. Following transfection, the treated segments were rinsed with 0.9% sodium chloride (3 x 100 µl) and upon cannula removal, the arteriotomy site was repaired with microsutures, restoring normal blood flow and the neck wound closed. All experimental protocols in this project were

approved by the Institutional Committee for Animal Protection of the Louis-Charles Simard Research Center.

Neointimal hyperplasia inhibition

A total of 36 New Zealand white rabbit carotid arteries were injured with a 2.5 mm balloon catheter serially inflated for 1 minute to 4, 6, 8 and 10 atm with gentle traction, allowing 45 seconds between inflations. Two weeks later, a second injury was imposed at the same arterial site which was then transfected in a 1 cm portion with 80 μmol/L (100 μl of volume injected) of therapeutic molecules or with 100 μL of NaCl 0.9% as control. Intimal/medial areas were evaluated by computer analysis on histological sections derived from transfected arteries two weeks following the second injury and transfection procedure.

The addition of a PC5 antisense 17-mer ODN shown in Table 1 at the time of a second carotid injury with a balloon catheter decreased carotid stenosis, measured as area ratio intima/media, by 40% (Area ratio intima/media <u>sense</u> ODN minus area ratio <u>antisense</u> ODN divided by area ratio sense ODN; see figure 9). This inhibitory effect is highly significant ($P = 0.0118$ and $P = 0.0078$) as compared to the sense and random controls, respectively. These results are the basis of a method of preventing stenosis comprising administering an effective stenosis inhibitory dose of a PC5 antisense to a subject in need for such a treatment. Other antisense oligonucleotides may be added to optimize this method of preventing restenosis, such as those silencing the expression of other convertases, namely PC2, which are also observed in atherectomy specimens (see Figure 10).

The development of drugs based on the inhibition or the inactivation of the convertases is of great interest because the drugs can easily be delivered directly at the affected site during the intervention by the cardiologist.

In addition we claim that this therapeutic approach, based on the inhibition of cell growth by antisense against one of the convertases will be applicable to all

proliferative diseases involving maturation of a given proteic precursor into an active protein.

TABLE 1

The sequences of the oligonucleotides used are:

Antisense	GCAACTTGCCAGAGCAT	SEQ ID NO: 3
Sense	ATGCTCTGGCAAGTTGC	
Random	AATCCGTGAGACCAGTC.	

PC5 is involved in the cleavage of HIV gp160 into gp120 and gp41.

As mentioned above, PC5, PC7 and furin are known to be present in CD4$^+$ T lymphocytes. All three enzymes cleave HIV gp160 to gp120 and gp41 as well as a synthetic peptide covering the junction wherein cleavage occurs in gp160. Since the 17-mer antisense ODN defined in Seq ID. No. 3 successfully silenced the expression of PC5 and prevented restenosis, the same oligonucleotide as well as any other oligonucleotide or construct having an equivalent silencing function will find use in inhibiting the action of PC5 on HIV gp160 in CD4$^+$ T lymphocytes. To optimize the inhibition of conversion of gp160 into its fusiogenic form, a cocktail comprising antisense molecules to PC5, PC7 and furin is contemplated as part of the present invention. Appropriate vehicles such as liposomes may be used to deliver these antisense molecules to the target tissues.

This invention has been described hereinbelow. It may be apparent to the skilled reader that modifications can be made thereto without departing from the above teachings. These modifications are considered as part of the scope of the present invention, as defined in the appended claims.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A purified human pro-protein converting enzyme named PC5.

2. A purified human pro-protein converting enzyme named PC5 isolated from adrenals.

3. A pro-protein converting enzyme as defined in claim 2 which has the amino acid sequence of SEQ ID NO: 2.

4. An isolated nucleic acid encoding the pro-protein converting enzyme defined in claim 1, 2 or 3.

5. An isolated nucleic acid as defined in claim 4 which has the nucleic acid sequence of SEQ ID NO: 1.

6. An oligonucleotide having at least 15 nucleotides which is a part of the nucleic defined in claim 4 or 5 or its complementary sequence.

7. An oligonucleotide which has the nucleic acid sequence defined in SEQ ID NO: 3.

8. A method of converting a proteic precursor cleavable by PC5 into a mature protein, which comprises the steps of contacting said protein precursor with the pro-protein converting enzyme of claim 1 2 or 3 in conditions supporting the activity of said enzyme and recovering said protein.

9. A method of inhibiting muscle cell proliferation which comprises the steps of contacting muscle cells with the oligonucleotide of claim 7 or any equivalent silencing antisense molecule.

10. A method as defined in claim 9, which results in the prevention of restenosis.

11. A method of silencing the expression of PC5 in a cell, which comprises the step of contacting said cell with the complementary sequence of the nucleic acid defined in claim 4 or 5, or a part thereof having at least 15 nucleotides.

12. A method as defined in claim 8, wherein said precursor is HIV gp160.

13. A method as defined in claim 11, wherein said cell is $CD4^+$ T lymphocyte.

14. A method as defined in claim 13, wherein said cell is HIV infected T lymphocyte.

15. A method as defined in claim 14, which results in inhibition of cleavage of HIV gp160 by PC5.

16. A method as defined in any one of claims 11, and 13 to 15, wherein said part of said complementary sequence comprises the oligonucleotide defined in SEQ ID NO: 3.

17. A method as defined in claim 15, wherein said part of said complementary sequence comprises the oligonucleotide defined in SEQ ID NO: 3.

18. A method as defined in claim 17, further comprising antisense silencing molecules to PC7 and furin.

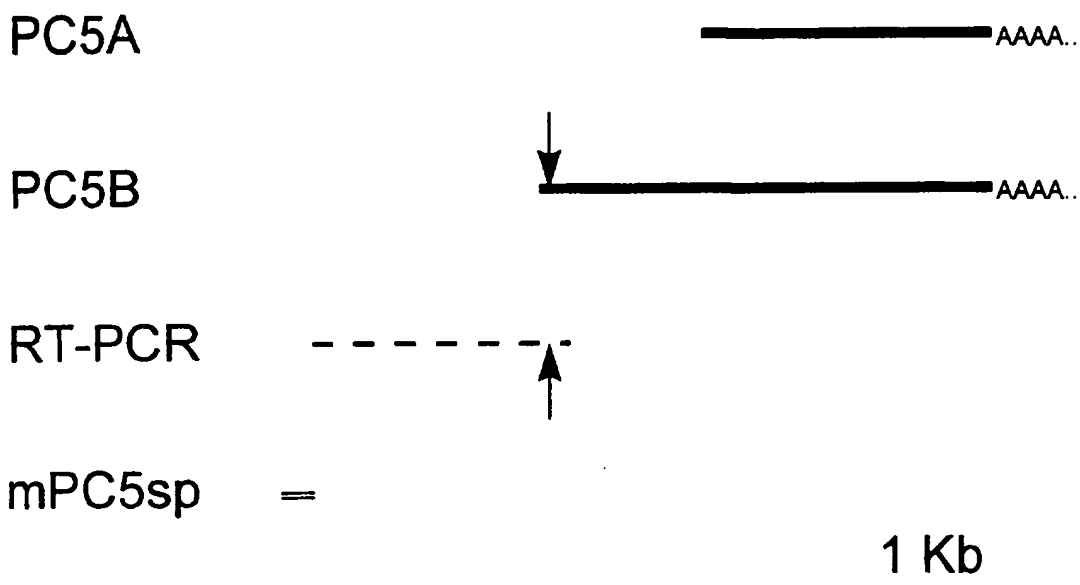

FIG. 1

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ATGGACTGGGACTGGGGGAACCGCTGCAGCCGCCCGGGACGGCGGGATCTGCTGTGCGTGCTGGCGCTGCTCGGGGGCTGCCTGCTCCCCGTGTGTCGGACGCGCGTCTACACCAACCAC 120
M D W D W G N R C S R P G R R D L L C V L A L L G G C L L P V C R T R V Y T N H

TGGGCAGTCAAAATCGCCGGGGGCTTCCCGGAGGCCAACCGTATCGCCAGCAAGTACGGATTCATCAACATAGGACAGATAGGGGCCCTGAAGGACTACTACCACTTCTACCATAGCAGG 240
W A V K I A G G F P E A N R I A S K Y G F I N I G Q I G A L K D Y Y H F Y H S R

ACGATTAAAAGGTCAGTTATCTCGAGCAGAGGGACCCACAGTTTCATTTCAATGGAACCAAAGGTGGAATGGATCCAACAGCAAGTGGTAAAAAAGCGGACAAAGAGGGATTATGACTTC 360
T I K R S V I S S R G T H S F I S M E P K V E W I Q Q Q V V K K R T K R D Y D F

AGTCGTGCCCAGTCTACCTATTTCAATGATCCCAAGTGGCCCAGCATGTGGTATATGCACTGCAGTGACAATACACATCCCTGCCAGTCTGACATGAATATCGAAGGAGCCTGGAAGAGA 480
S R A Q S T Y F N D P K W P S M W Y M H C S D N T H P C Q S D M N I E G A W K R

GGCTACACGGGAAAGAACATTGTGGTCACTATCCTGGATGACGGAATTGAGAGAACCCATCCAGATCTGATGCAAAACTACGATGCTCTGGCAAGTTGCGACGTGAATGGGAATGACTTG 600
G Y T G K N I V V T I L D D G I E R T H P D L M Q N Y D A L A S C D V N G N D L

GACCCAATGCCTCGTTATGATGCAAGCAACGAGAACAAGCATGGGACTCGCTGTGCTGGAGAAGTGGCAGCCGCTGCAAACAATTCGCACTGCACAGTCGGAATTGCTTTCAACGCCAAG 720
D P M P R Y D A S N E N K H G T R C A G E V A A A A N N S H C T V G I A F N A K

ATCGGAGGAGTGCGAATGCTGGACGGAGATGTCACGGACATGGTTGAAGCAAAATCAGTTAGCTTCAACCCCCAGCACGTGCACATTTACAGCGCCAGCTGGGGCCCGGATGATGATGGC 840
I G G V R M L D G D V T D M V E A K S V S F N P Q H V H I Y S A S W G P D D D G

AAGACTGTGGACGGACCAGCCCCCCTCACCCGGCAAGCCTTTGAAAACGGCGTTAGAATGGGGCGGAGAGGCCTCGGCTCTGTGTTTGTTTGGGCATCTGGAAATGGTGGAAGGAGCAAA 960
K T V D G P A P L T R Q A F E N G V R M G R R G L G S V F V W A S G N G G R S K

GACCACTGCTCCTGTGATGGCTACACCAACAGCATCTACACCATCTCCATCAGCAGCACTGCAGAAAGCGGAAAGAAACCTTGGTACCTGGAAGAGTGTTCATCCACGCTGGCCACAACC 1080
D H C S C D G Y T N S I Y T I S I S S T A E S G K K P W Y L E E C S S T L A T T

TACAGCAGCGGGGAGTCCTACGATAAGAAAATCATCACTACAGATCTGAGGCAGCGTTGCACGGACAACCACACTGGGACGTCAGCCTCAGCCCCCATGGCTGCAGGCATCATTGCGCTG 1140
Y S S G E S Y D K K I I T T D L R Q R C T D N H T G T S A S A P M A A G I I A L

GCCCTGGAAGCCAATCCGTTTCTGACCTGGAGAGACGTACAGCATGTTATTGTCAGGACTTCCCGTGCGGGACATTTGAACGCTAATGACTGGAAAACCAATGCTGCTGGTTTTAAGGTG 1320
A L E A N P F L T W R D V Q H V I V R T S R A G H L N A N D W K T N A A G F K V

AGCCATCTTTATGGATTTGGACTGATGGACGCAGAAGCCATGGTGATGGAGGCAGAGAAGTGGACCACCGTTCCCCGGCAGCACGTGTGTGTGGAGAGCACAGACCGACAAATCAAGACA 1440
S H L Y G F G L M D A E A M V M E A E K W T T V P R Q H V C V E S T D R Q I K T

FIG. 2A

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ATCCGCCCTAACAGTGCAGTGCGCTCCATCTACAAAGCTTCAGGCTGCTCGGATAACCCCAACCGCCATGTCAACTACCTGGAGCACGTCGTTGTGCGCATCACCATCACCCACCCCAGG 1560
 I  R  P  N  S  A  V  R  S  I  Y  K  A  S  G  C  S  D  N  P  N  R  H  V  N  Y  L  E  H  V  V  V  R  I  T  I  T  H  P  R

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 R  G  D  L  A  I  Y  L  T  S  P  S  G  T  R  S  Q  L  L  A  N  R  L  F  D  H  S  M  E  G  F  K  N  W  E  F  M  T  I  H

TGCTGGGGAGAAAGAGCTGCTGGTGACTGGGTCCTTGAAGTTTATGATACTCCCTCTCAGCTAAGGAACTTTAAGACTCCAGGTAAATTGAAAGAATGGTCTTTGGTCCTCTACGGCACC 1800
 C  W  G  E  R  A  A  G  D  W  V  L  E  V  Y  D  T  P  S  Q  L  R  N  F  K  T  P  G  K  L  K  E  W  S  L  V  L  Y  G  T

TCCGTGCAGCCATATTCACCAACCAATGAATTTCCGAAAGTGGAACGGTTCCGCTATAGCCGAGTTGAAGACCCCACAGACGACTATGGCACAGAGGATTATGCAGGTCCCTGCGACCCT 1920
 S  V  Q  P  Y  S  P  T  N  E  F  P  K  V  E  R  F  R  Y  S  R  V  E  D  P  T  D  D  Y  G  T  E  D  Y  A  G  P  C  D  P

GAGTGCAGTGAGGTTGGCTGTGACGGGCCAGGACCAGACCACTGCAATGACTGTTTGCACTACTACTACAAGCTGAAAAACAATACCAGGATCTGTGTCTCCAGCTGCCCCCCTGGCCAC 2040
 E  C  S  E  V  G  C  D  G  P  G  P  D  H  C  N  D  C  L  H  Y  Y  Y  K  L  K  N  N  T  R  I  C  V  S  S  C  P  P  G  H

TACCACGCCGACAAGAAGCGCTGCAGGAAGTGTGCCCCCAACTGTGAGTCCTGCTTTGGGAGCCATGGTGACCAATGCATGTCCTGCAAATATGGATACTTTCTGAATGAAGAAACCAAC 2160
 Y  H  A  D  K  K  R  C  R  K  C  A  P  N  C  E  S  C  F  G  S  H  G  D  Q  C  M  S  C  K  Y  G  Y  F  L  N  E  E  T  N

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GGGTTAAGCCTGCAGGGATCCCGGTGCTCTGTCTCCTGTGAAGATGGACGGTATTTCAACGGCCAGGACTGCCAGCCCTGCCACCGCTTCTGCGCCACTTGTGCTGGGGCAGGAGCTGAT 2400
 G  L  S  L  Q  G  S  R  C  S  V  S  C  E  D  G  R  Y  F  N  G  Q  D  C  Q  P  C  H  R  F  C  A  T  C  A  G  A  G  A  D

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GATATCAGTTGTTTGACGTGCAATGGCCCAGGATTCAAGAACTGTACAAGCTGCCCTAGTGGGTATCTCTTAGACTTAGGAATGTGTCAAATGGGAGCCATTTGCAAGGATGCAACGGAA 2640
 D  I  S  C  L  T  C  N  G  P  G  F  K  N  C  T  S  C  P  S  G  Y  L  L  D  L  G  M  C  Q  M  G  A  I  C  K  D  A  T  E

GAGTCCTGGGCGGAAGGAGGCTTCTGTATGCTTGTGAAAAAGAACAATCTGTGCCAACGGAAGGTTCTTCAACAACTTTGCTGCAAAACATGTACATTTCAAGGCTGAGCAGCCATCTTA 2760
 E  S  W  A  E  G  G  F  C  M  L  V  K  K  N  N  L  C  Q  R  K  V  L  Q  Q  L  C  C  K  T  C  T  F  Q  G STOP

GATTTCTTTGTTCCTGTAGACTTATAGATTATTCCATATTATTAAAAAGAAAAAAAAAA
```

FIG. 2B

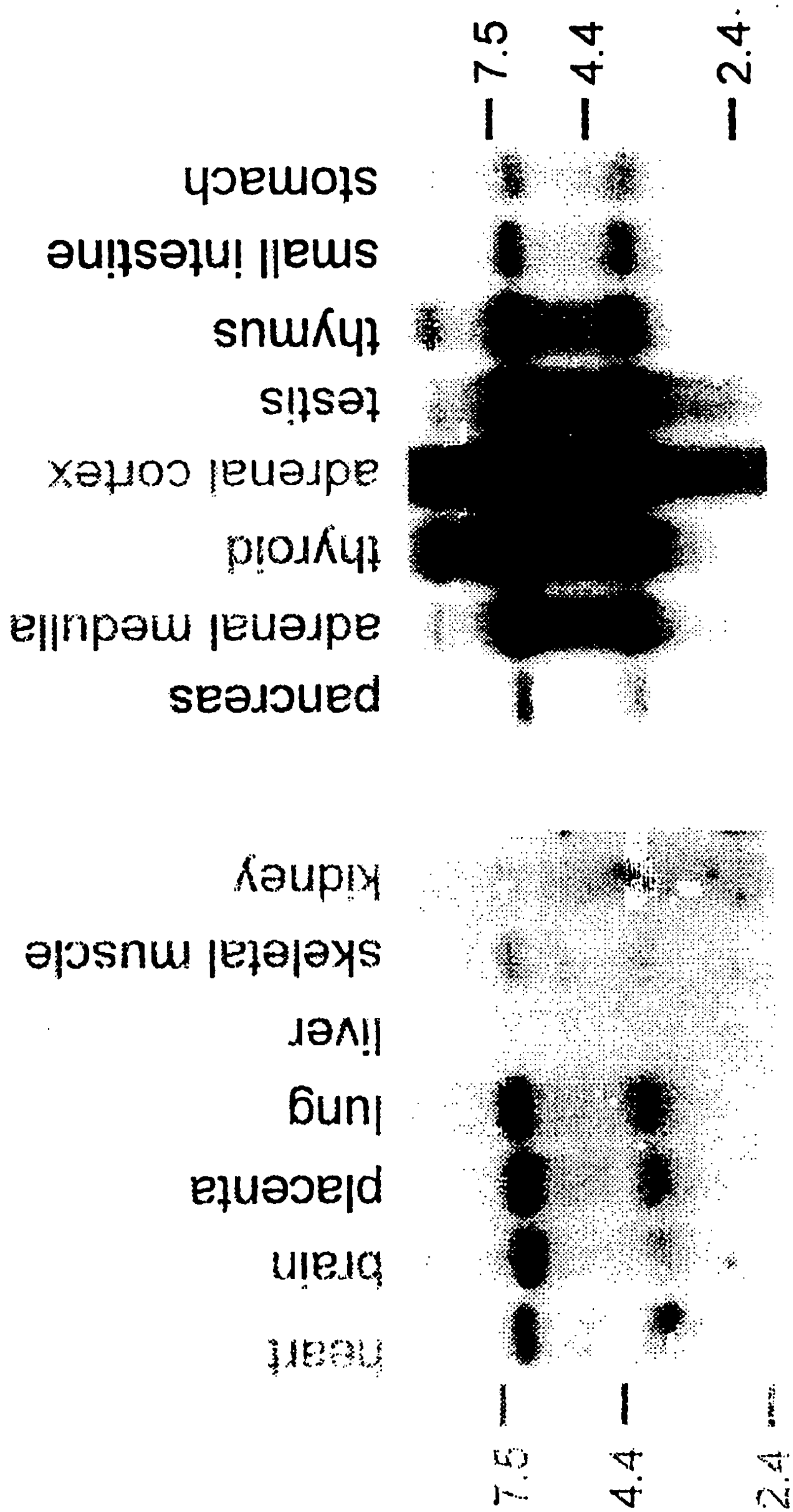
stomach
small intestine
thymus
testis
adrenal cortex
thyroid
adrenal medulla
pancreas
kidney
skeletal muscle
liver
lung
placenta
brain
heart
7.5
4.4
2.4

FIG. 3

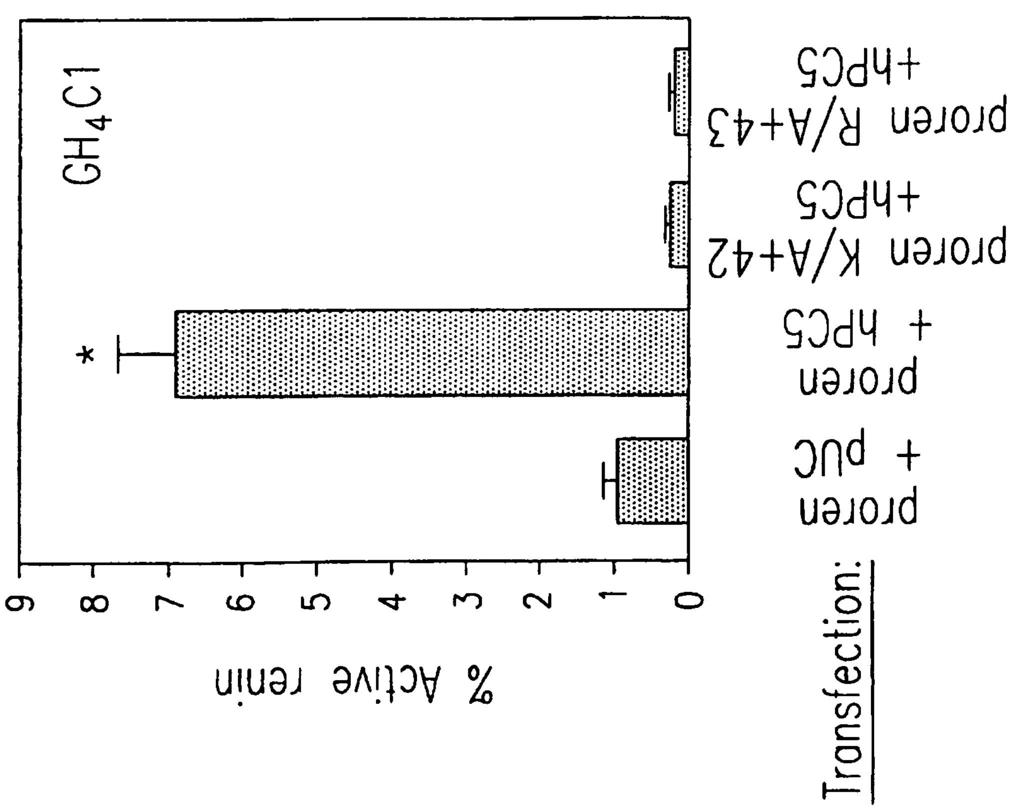
GH$_4$C1
% Active renin
0
1
2
3
4
5
6
7
8
9
*
Transfection:
proren + pUC
proren + hPC5
proren K/A+42 +hPC5
proren R/A+43 +hPC5

FIG. 4A

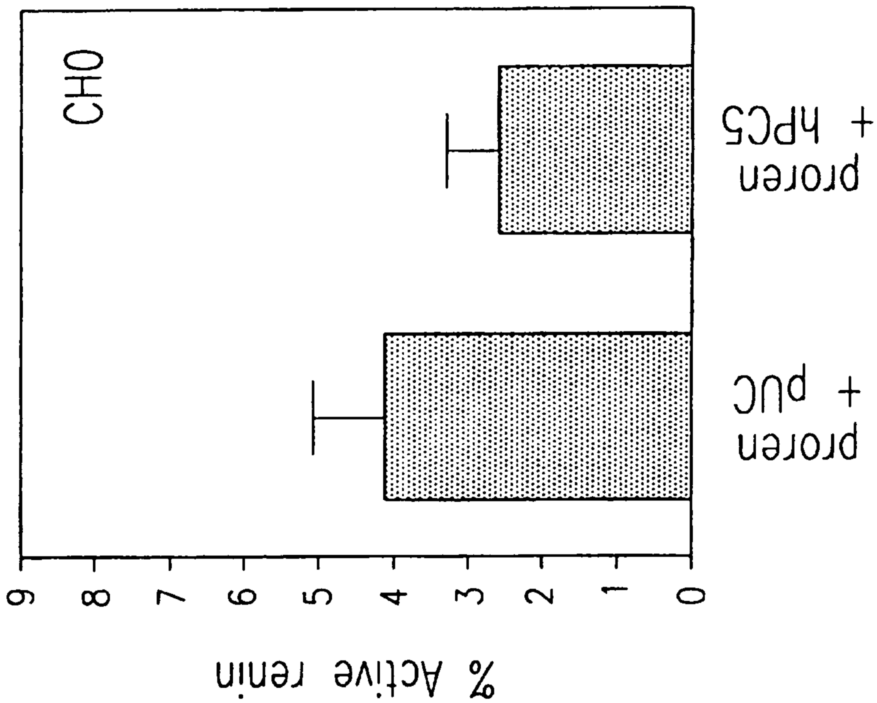
CHO
% Active renin
0
1
2
3
4
5
6
7
8
9
proren + pUC
proren + hPC5

FIG. 4B

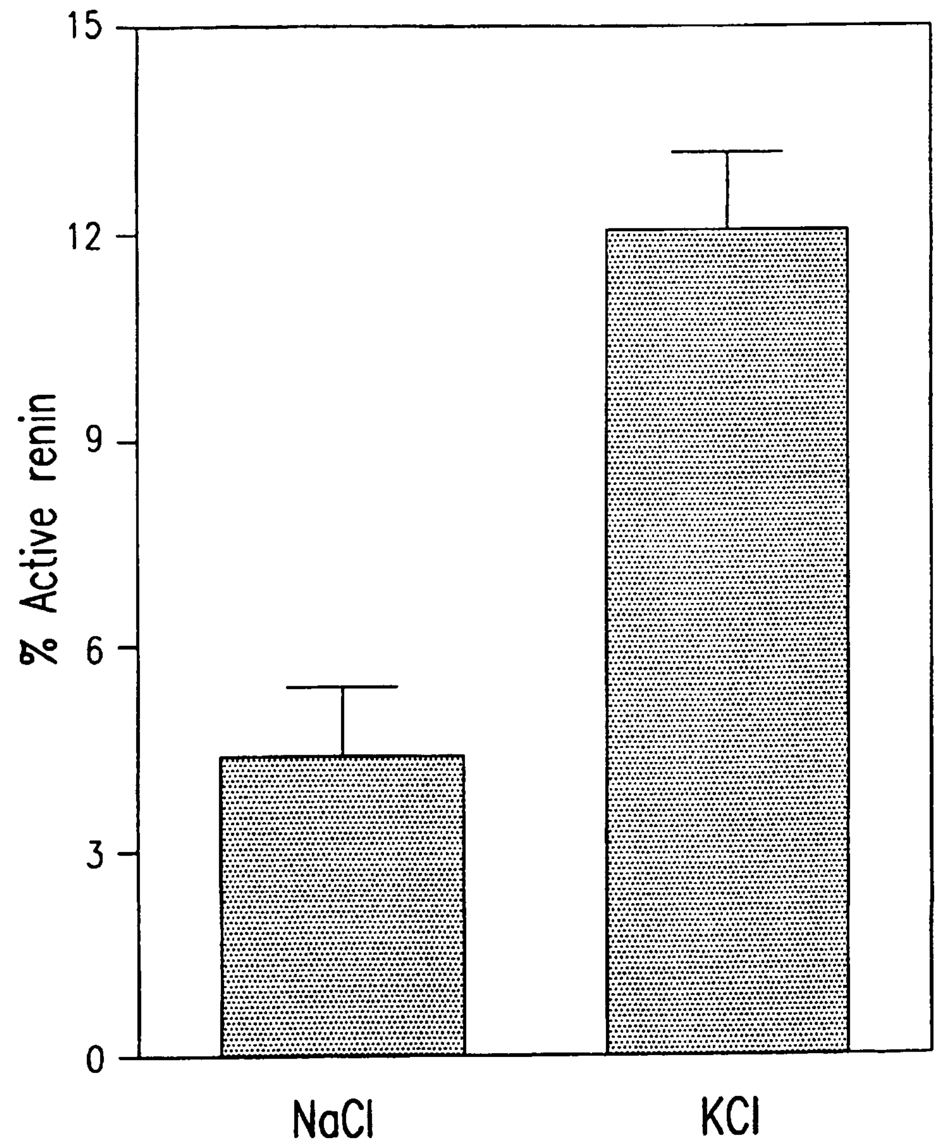

FIG. 5

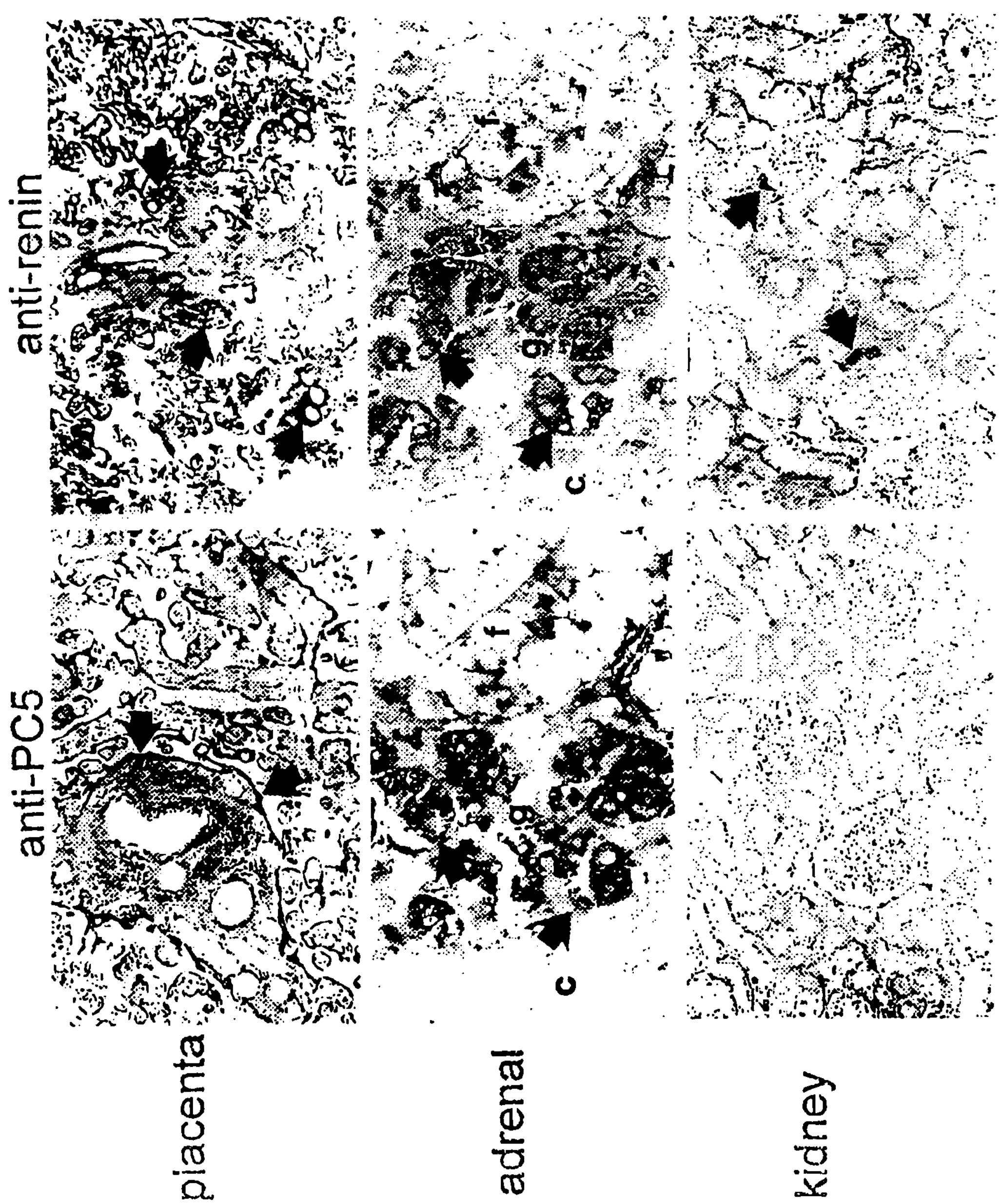

FIG. 6

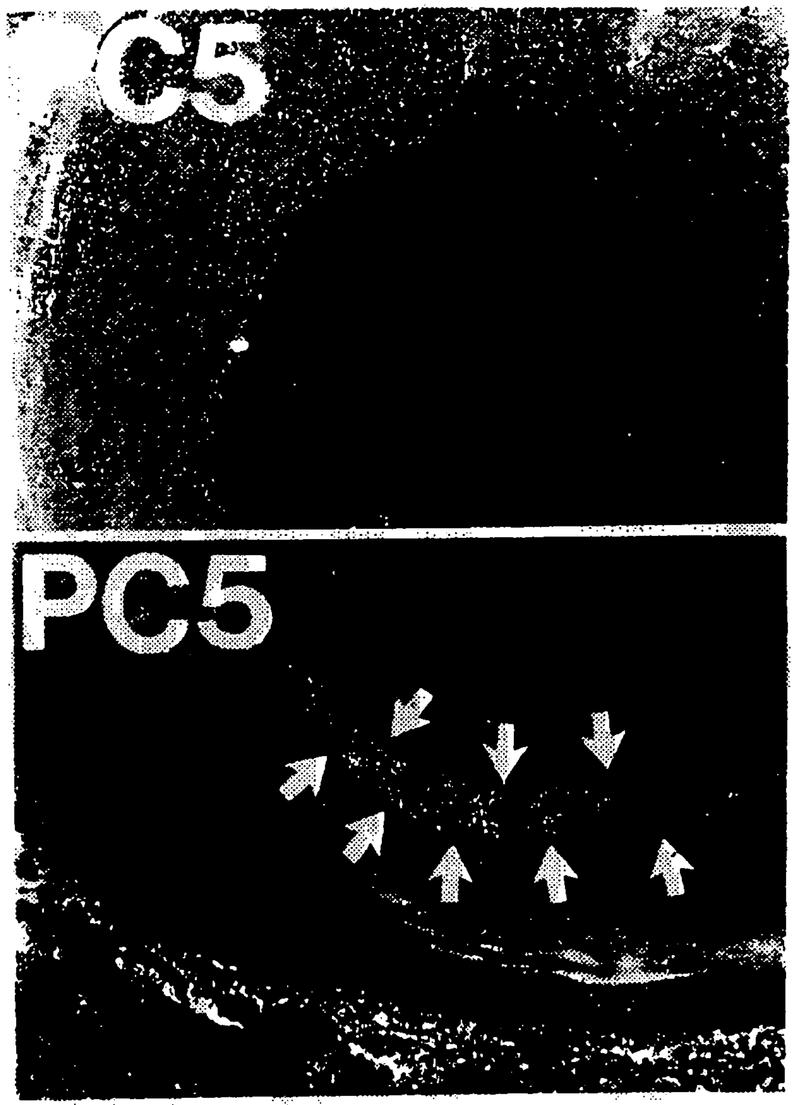

FIG. 7

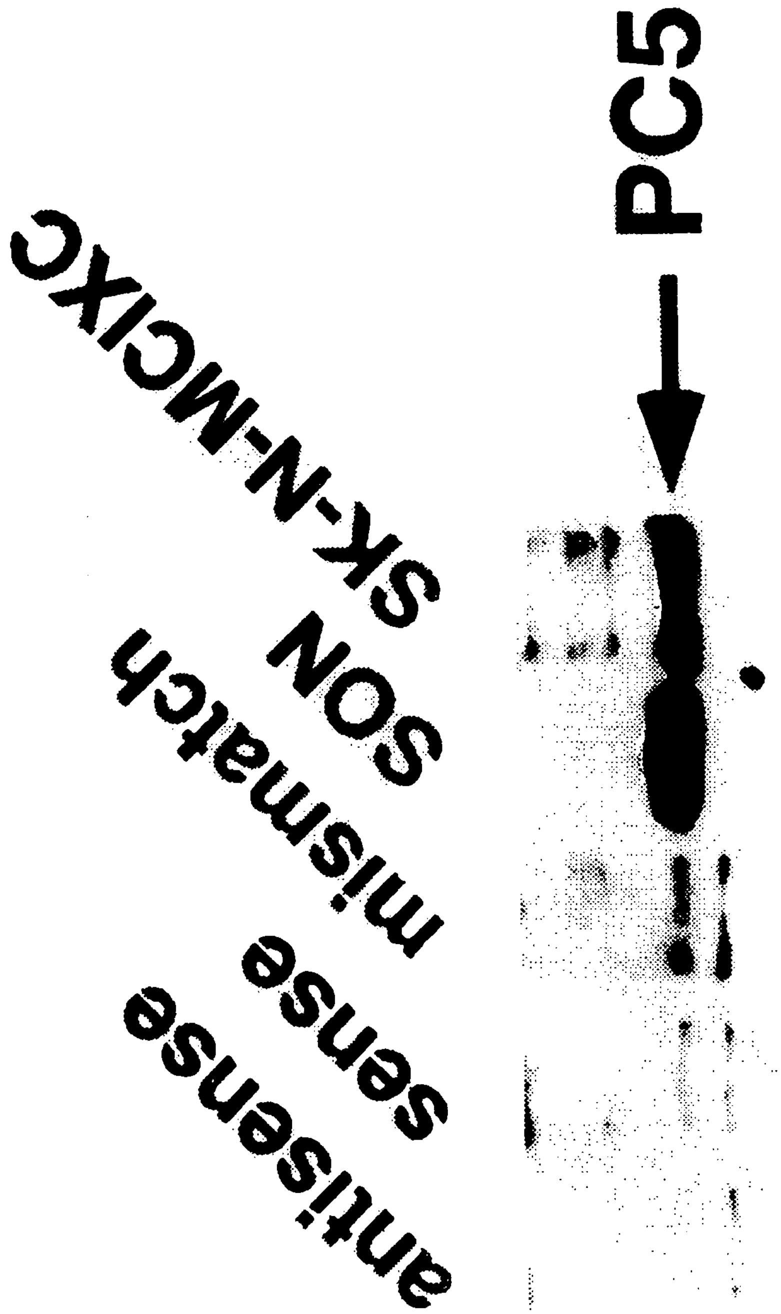

FIG. 8

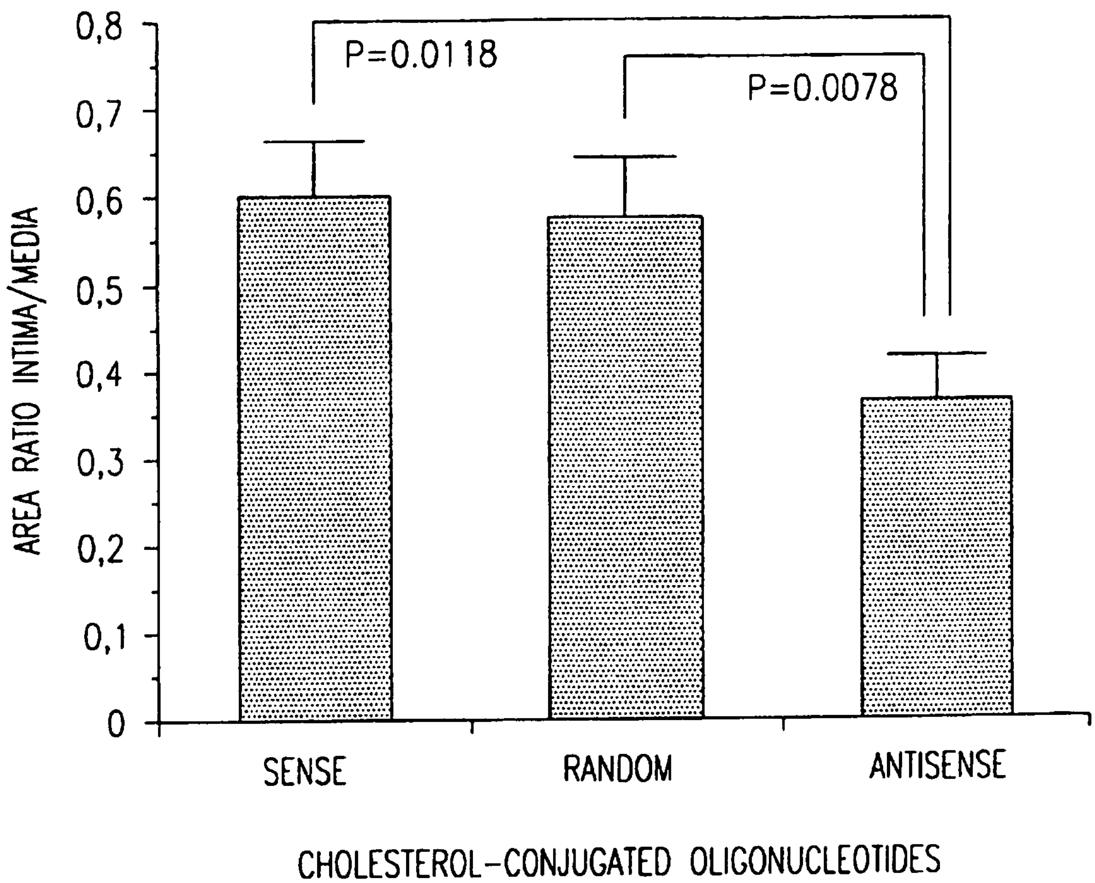

FIG. 9

	PC1	PC2	PC5	PC7	Furin
Number of Slide	n=31	n=29	n=25	n=27	n=30
Mean Positivity (Scale 0-3)	0.29	2.03	2.08	1.19	1.08
SD	0.46	0.61	0.62	1.04	1.07
% (+) Slide	29.0	96.6	96.0	63.0	60

FIG. 10

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```
Miranda1 AGCGTCGGGACCATGGATTGGGATTGGGGGAACCGCTGCAGCCGCCCGGG 50
                     ||||| ||||| ||||||||||||||||||||||||||
IRCM   1 ............ATGGACTGGGACTGGGGGAACCGCTGCAGCCGCCCGGG 38

      51 ACGGCGGACCTGCTGTGCGTGCTGGCACTGCTCGCCGGCTGTCTGCTCC 100
         |||||||| ||||||||||||||||| |||||||  |||||| |||||||
      39 ACGGCGGATCTGCTGTGCGTGCTGGCGCTGCTCGGGGGCTGCCTGCTCC 88

     101 CGGTATGCCGGACGCGCGTCTACACCAACCACTGGCAGTGAAGATCGCC 150
         | || || ||||||||||||||||||||||||||||||| || ||||||
      89 CCGTGTGTCGGACGCGCGTCTACACCAACCACTGGCAGTCAAAATCGCC 138

     151 GGCGGCTTCGCGGAGGCAGATCGCATAGCCAGCAAGTACGGATTCATCAA 200
         || |||||| |||||||  | || ||  |||||||||||||||||||||||
     139 GGGGGCTTCCCGGAGGCCAACCGTATCGCCAGCAAGTACGGATTCATCAA 188

     201 CGTAGGACAGATCGGTGCACTGAAGGACTACTATCACTTCTACCATAGTA 250
         | ||||||||||| || || ||||||||||||||| |||||||||||||| |
```

FIG. 11A

```
189 CATAGGACAGATAGGGGCCCTGAAGGACTACTACCACTTCTACCATAGCA 238
             .         .         .         .         .
251 GGACCATTAAAAGGTCTGTTCTCTCGAGCAGAGGAACCCACAGTTTCATT 300
    |||| ||||||||||| ||| ||||||||||||| |||||||||||||||
239 GGACGATTAAAAGGTCAGTTATCTCGAGCAGAGGGACCCACAGTTTCATT 288
             .         .         .         .         .
301 TCAATGGAACCAAAGGTGGAGTGGATCCAACAGCAAGTGGTGAAAAAAAG 350
    |||||||||||||||||||| |||||||||||||||||||| |||||  |
289 TCAATGGAACCAAAGGTGGAATGGATCCAACAGCAAGTGGTAAAAAAGCG 338
             .         .         .         .         .
351 AACCAAGAGGGATTATGACCTCAGCCATGCCCAGTCAACCTACTTCAATG 400
     || ||||||||||||||| |||| | ||||||||| ||||| |||||||
339 GACAAAGAGGGATTATGACTTCAGTCGTGCCCAGTCTACCTATTTCAATG 388
             .         .         .         .         .
401 ATCCCAAGTGGCCAAGTATGTGGTACATGCACTGTAGCGACAATACACAT 450
    ||||||||||||| || |||||||| |||||||| || ||||||||||||
389 ATCCCAAGTGGCCCAGCATGTGGTATATGCACTGCAGTGACAATACACAT 438
             .         .         .         .         .
451 CCCTGCCAGTCTGACATGAATATCGAAGGAGCCTGGAAGAGAGGCTACAC 500
    ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11B

```
439 CCCTGCCAGTCTGACATGAATATCGAAGGAGCCTGGAAGAGAGGCTACAC 488
              .         .         .         .         .
501 GGGAAAGAACATTGTGGTCACTATCCTGGATGACGGAATTGAGAGAACCC 550
    ||||||||||||||||||||||||||||||||||||||||||||||||||
489 GGGAAAGAACATTGTGGTCACTATCCTGGATGACGGAATTGAGAGAACCC 538
              .         .         .         .         .
551 ATCCAGATCTGATGCAAAACTACGATGCTCTGGCAAGTTGCGACGTGAAT 600
    ||||||||||||||||||||||||||||||||||||||||||||||||||
539 ATCCAGATCTGATGCAAAACTACGATGCTCTGGCAAGTTGCGACGTGAAT 588
              .         .         .         .         .
601 GGGAATGACTTGGACCCAATGCCTCGTTATGATGCAAGCAACGAGAACAA 650
    ||||||||||||||||||||||||||||||||||||||||||||||||||
589 GGGAATGACTTGGACCCAATGCCTCGTTATGATGCAAGCAACGAGAACAA 638
              .         .         .         .         .
651 GCATGGGACTCGCTGTGCTGGAGAAGTGGCAGCCGCTGCAAACAATTCGC 700
    ||||||||||||||||||||||||||||||||||||||||||||||||||
639 GCATGGGACTCGCTGTGCTGGAGAAGTGGCAGCCGCTGCAAACAATTCGC 688
              .         .         .         .         .
701 ACTGCACAGTCGGAATTGCTTTCAACGCCAAGATCGGAGGAGTGCGAATG 750
    ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11C

```
689 ACTGCACAGTCGGAATTGCTTTCAACGCCAAGATCGGAGGAGTGCGAATG 738
             .         .         .         .         .
751 CTGGACGGAGATGTCACGGACATGGTTGAAGCAAAATCAGTTAGCTTCAA 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
739 CTGGACGGAGATGTCACGGACATGGTTGAAGCAAAATCAGTTAGCTTCAA 788
             .         .         .         .         .
801 CCCCCAGCACGTGCACATTTACAGCGCCAGCTGGGGCCCGGATGATGATG 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
789 CCCCCAGCACGTGCACATTTACAGCGCCAGCTGGGGCCCGGATGATGATG 838
             .         .         .         .         .
851 GCAAGACTGTGGACGGACCAGCCCCCTCACCCGGCAAGCCTTTGAAAAC 900
    ||||||||||||||||||||||||||||||||||||||||||||||||||
839 GCAAGACTGTGGACGGACCAGCCCCCTCACCCGGCAAGCCTTTGAAAAC 888
             .         .         .         .         .
901 GGCGTTAGAATGGGGCGGAGAGGCCTCGGCTCTGTGTTTGTTTGGGCATC 950
    ||||||||||||||||||||||||||||||||||||||||||||||||||
889 GGCGTTAGAATGGGGCGGAGAGGCCTCGGCTCTGTGTTTGTTTGGGCATC 938
             .         .         .         .         .
951 TGGAAATGGTGGAAGGAGCAAAGACCACTGCTCCTGTGATGGCTACACCA 1000
    ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11D

```
 939 TGGAAATGGTGGAAGGAGCAAAGACCACTGCTCCTGTGATGGCTACACCA 988
               .         .         .         .         .
1001 ACAGCATCTACACCATCTCCATCAGCAGCACTGCAGAAAGCGGAAAGAAA 1050
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 989 ACAGCATCTACACCATCTCCATCAGCAGCACTGCAGAAAGCGGAAAGAAA 1038
               .         .         .         .         .
1051 CCTTGGTACCTGGAAGAGTGTTCATCCACGCTGGCCACAACCTACAGCAG 1100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1039 CCTTGGTACCTGGAAGAGTGTTCATCCACGCTGGCCACAACCTACAGCAG 1088
               .         .         .         .         .
1101 CGGGGAGTCCTACGATAAGAAAATCATCACTACAGATCTGAGGCAGCGTT 1150
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1089 CGGGGAGTCCTACGATAAGAAAATCATCACTACAGATCTGAGGCAGCGTT 1138
               .         .         .         .         .
1151 GCACGGACAACCACACTGGGACGTCAGCCTCAGCCCCCATGGCTGCAGGC 1200
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1139 GCACGGACAACCACACTGGGACGTCAGCCTCAGCCCCCATGGCTGCAGGC 1188
               .         .         .         .         .
1201 ATCATTGCGCTGGCCCTGGAAGCCAATCCGTTTCTGACCTGGAGAGACGT 1250
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11E

```
1189 ATCATTGCGCTGGCCCTGGAAGCCAATCCGTTTCTGACCTGGAGAGACGT 1238
               .         .         .         .         .
1251 ACAGCATGTTATTGTCAGGACTTCCCGTGCGGGACATTTGAACGCTAATG 1300
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1239 ACAGCATGTTATTGTCAGGACTTCCCGTGCGGGACATTTGAACGCTAATG 1288
               .         .         .         .         .
1301 ACTGGAAAACCAATGCTGCTGGTTTTAAGGTGAGCCATCTTTATGGATTT 1350
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1289 ACTGGAAAACCAATGCTGCTGGTTTTAAGGTGAGCCATCTTTATGGATTT 1338
               .         .         .         .         .
1351 GGACTGATGGACGCAGAAGCCATGGTGATGGAGGCAGAGAAGTGGACCAC 1400
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1339 GGACTGATGGACGCAGAAGCCATGGTGATGGAGGCAGAGAAGTGGACCAC 1388
               .         .         .         .         .
1401 CGTTCCCCGGCAGCACGTGTGTGGAGAGCACAGACCGACAAATCAAGA 1450
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1389 CGTTCCCCGGCAGCACGTGTGTGGAGAGCACAGACCGACAAATCAAGA 1438
               .         .         .         .         .
1451 CAATCCGCCCTAACAGTGCAGTGCGCTCCATCTACAAAGCTTCAGGCTGC 1500
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11F

```
1439 CAATCCGCCCTAACAGTGCAGTGCGCTCCATCTACAAAGCTTCAGGCTGC 1488
              .         .         .         .         .
1501 TCGGATAACCCCAACCGCCATGTCAACTACCTGGAGCACGTCGTTGTGCG 1550
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1489 TCGGATAACCCCAACCGCCATGTCAACTACCTGGAGCACGTCGTTGTGCG 1538
              .         .         .         .         .
1551 CATCACCATCACCCACCCCAGGAGAGGAGACCTGGCCATCTACCTGACCT 1600
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1539 CATCACCATCACCCACCCCAGGAGAGGAGACCTGGCCATCTACCTGACCT 1588
              .         .         .         .         .
1601 CGCCCTCTGGAACTAGGTCTCAGCTTTTGGCCAACAGGCTATTTGATCAC 1650
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1589 CGCCCTCTGGAACTAGGTCTCAGCTTTTGGCCAACAGGCTATTTGATCAC 1638
              .         .         .         .         .
1651 TCCATGGAAGGATTCAAAAACTGGGAGTTCATGACCATTCATTGCTGGGG 1700
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1639 TCCATGGAAGGATTCAAAAACTGGGAGTTCATGACCATTCATTGCTGGGG 1688
              .         .         .         .         .
1701 AGAAAGAGCTGCTGGTGACTGGGTCCTTGAAGTTTATGATACTCCCTCTC 1750
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11G

```
1689 AGAAAGAGCTGCTGGTGACTGGGTCCTTGAAGTTTATGATACTCCCTCTC 1738
              .         .         .         .         .
1751 AGCTAAGGAACTTTAAGACTCCAGGTAAATTGAAAGAATGGTCTTTGGTC 1800
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1739 AGCTAAGGAACTTTAAGACTCCAGGTAAATTGAAAGAATGGTCTTTGGTC 1788
              .         .         .         .         .
1801 CTCTACGGCACCTCCGTGCGGCCATATTCACCAACCAATGAATTTCCGAA 1850
     ||||||||||||||||||| ||||||||||||||||||||||||||||||
1789 CTCTACGGCACCTCCGTGCAGCCATATTCACCAACCAATGAATTTCCGAA 1838
              .         .         .         .         .
1851 AGTGGAACGGTTCCGCTATAGCCGAGTTGAAGACCCCACAGACGACTATG 1900
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1839 AGTGGAACGGTTCCGCTATAGCCGAGTTGAAGACCCCACAGACGACTATG 1888
              .         .         .         .         .
1901 GCACAGAGGATTATGCAGGTCCCTGCGACCCTGAGTGCAGTGAGGTTGGC 1950
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1889 GCACAGAGGATTATGCAGGTCCCTGCGACCCTGAGTGCAGTGAGGTTGGC 1938
              .         .         .         .         .
1951 TGTGACGGGCCAGGACCAGACCACTGCAATGACTGTTTGCACTACTACTA 2000
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11H

```
1939 TGTGACGGGCCAGGACCAGACCACTGCAATGACTGTTTGCACTACTACTA 1988
                .         .         .         .         .
2001 CAAGCTGAAAAACAATACCAGGATCTGTGTCTCCAGCTGCCCCCCTGGCC 2050
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1989 CAAGCTGAAAAACAATACCAGGATCTGTGTCTCCAGCTGCCCCCCTGGCC 2038
                .         .         .         .         .
2051 ACTACCACGCCGACAAGAAGCGCTGCAGGAAGTGTGCCCCCAACTGTGAG 2100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2039 ACTACCACGCCGACAAGAAGCGCTGCAGGAAGTGTGCCCCCAACTGTGAG 2088
                .         .         .         .         .
2101 TCCTGCTTTGGGAGCCATGGTGACCAATGCATGTCCTGCAAATATGGATA 2150
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2089 TCCTGCTTTGGGAGCCATGGTGACCAATGCATGTCCTGCAAATATGGATA 2138
                .         .         .         .         .
2151 CTTTCTGAATGAAGAAACCAACAGCTGTGTTACTCACTGCCCTGATGGGT 2200
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2139 CTTTCTGAATGAAGAAACCAACAGCTGTGTTACTCACTGCCCTGATGGGT 2188
                .         .         .         .         .
2201 CATATCAGGATACCAAGAAAAATCTTTGCCGGAAATGCAGTGAAAACTGC 2250
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

Fig - 11I

```
2189 CATATCAGGATACCAAGAAAAATCTTTGCCGGAAATGCAGTGAAAACTGC 2238
              .         .         .         .         .
2251 AAGACATGTACTGAATTCCATAACTGTACAGAATGTAGGGATGGGTTAAG 2300
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2239 AAGACATGTACTGAATTCCATAACTGTACAGAATGTAGGGATGGGTTAAG 2288
              .         .         .         .         .
2301 CCTGCAGGGATCCCGGTGCTCTGTCTCCTGTGAAGATGGACGGTATTTCA 2350
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2289 CCTGCAGGGATCCCGGTGCTCTGTCTCCTGTGAAGATGGACGGTATTTCA 2338
              .         .         .         .         .
2351 ACGGCCAGGACTGCCAGCCCTGCCACCGCTTCTGCGCCACTTGTGCTGGG 2400
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2339 ACGGCCAGGACTGCCAGCCCTGCCACCGCTTCTGCGCCACTTGTGCTGGG 2388
              .         .         .         .         .
2401 GCAGGAGCTGATGGGTGCATTAACTGCACAGAGGGCTACTTCATGGAGGA 2450
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2389 GCAGGAGCTGATGGGTGCATTAACTGCACAGAGGGCTACTTCATGGAGGA 2438
              .         .         .         .         .
2451 TGGGAGATGCGTGCAGAGCTGTAGTATCAGCTATTACTTTGACCACTCTT 2500
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11J

```
2439 TGGGAGATGCGTGCAGAGCTGTAGTATCAGCTATTACTTTGACCACTCTT 2488
               .         .         .         .         .
2501 CAGAGAATGGATACAAATCCTGCAAAAAATGTGATATCAGTTGTTTGACG 2550
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2489 CAGAGAATGGATACAAATCCTGCAAAAAATGTGATATCAGTTGTTTGACG 2538
               .         .         .         .         .
2551 TGCAATGGCCCAGGATTCAAGAACTGTACAAGCTGCCCTAGTGGGTATCT 2600
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2539 TGCAATGGCCCAGGATTCAAGAACTGTACAAGCTGCCCTAGTGGGTATCT 2588
               .         .         .         .         .
2601 CTTAGACTTAGGAATGTGTCAAATGGGAGCCATTTGCAAGGATGCAACGG 2650
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2589 CTTAGACTTAGGAATGTGTCAAATGGGAGCCATTTGCAAGGATGCAACGG 2638
               .         .         .         .         .
2651 AAGAGTCCTGGGCGGAAGGAGGCTTCTGTATGCTTGTGAAAAAGAACAAT 2700
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2639 AAGAGTCCTGGGCGGAAGGAGGCTTCTGTATGCTTGTGAAAAAGAACAAT 2688
               .         .         .         .         .
2701 CTGTGCCAACGGAAGGTTCTTCAACAACTTTGCTGCAAAACATGTACATT 2750
     ||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG. 11K

```
2689 CTGTGCCAACGGAAGGTTCTTCAACAACTTTGCTGCAAAACATGTACATT 2738
              .         .         .         .         .
2751 CCAAGGCTGAGCAGCC.................................. 2766
      |||||||||||||||
2739 TCAAGGCTGAGCAGCCATCTTAGATTTCTTTGTTCCTGTAGACTTATAGA 2788
                               .
                               .
                               .
```

FIG. 11L

```
                  .         .         .         .         .
Mirandal   MDWDWGNRCSRPGRRDLLCVLALLAGCLLPVCRTRVYTNHWAVKIAGGFA  50
           |||||||||||||||||||||||| ||||||||||||||||||||||||
IRCM     1 MDWDWGNRCSRPGRRDLLCVLALLGGCLLPVCRTRVYTNHWAVKIAGGFP  50

                    .         .         .         .         .
        51 EADRIASKYGFINVGQIGALKDYYHFYHSRTIKRSVLSSRGTHSFISMEP 100
           ||.||||||||||:||||||||||||||||||||||:|||||||||||||
        51 EANRIASKYGFINIGQIGALKDYYHFYHSRTIKRSVISSRGTHSFISMEP 100

                    .         .         .         .         .
       101 KVEWIQQQVVKKRTKRDYDLSHAQSTYFNDPKWPSMWYMHCSDNTHPCQS 150
           ||||||||||||||||||| | ||||||||||||||||||||||||||||
       101 KVEWIQQQVVKKRTKRDYDFSRAQSTYFNDPKWPSMWYMHCSDNTHPCQS 150

                    .         .         .         .         .
       151 DMNIEGAWKRGYTGKNIVVTILDDGIERTHPDLMQNYDALASCDVNGNDL 200
           ||||||||||||||||||||||||||||||||||||||||||||||||||
       151 DMNIEGAWKRGYTGKNIVVTILDDGIERTHPDLMQNYDALASCDVNGNDL 200

                    .         .         .         .         .
       201 DPMPRYDASNENKHGTRCAGEVAAAANNSHCTVGIAFNAKIGGVRMLDGD 250
           ||||||||||||||||||||||||||||||||||||||||||||||||||
       201 DPMPRYDASNENKHGTRCAGEVAAAANNSHCTVGIAFNAKIGGVRMLDGD 250
```

FIG. 12A

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```
                     .         .         .         .         .
251 VTDMVEAKSVSFNPQHVHIYSASWGPDDDGKTVDGPAPLTRQAFENGVRM 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
251 VTDMVEAKSVSFNPQHVHIYSASWGPDDDGKTVDGPAPLTRQAFENGVRM 300
                     .         .         .         .         .
301 GRRGLGSVFVWASGNGGRSKDHCSCDGYTNSIYTISISSTAESGKKPWYL 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 GRRGLGSVFVWASGNGGRSKDHCSCDGYTNSIYTISISSTAESGKKPWYL 350
                     .         .         .         .         .
351 EECSSTLATTYSSGESYDKKIITTDLRQRCTDNHTGTSASAPMAAGIIAL 400
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 EECSSTLATTYSSGESYDKKIITTDLRQRCTDNHTGTSASAPMAAGIIAL 400
                     .         .         .         .         .
401 ALEANPFLTWRDVQHVIVRTSRAGHLNANDWKTNAAGFKVSHLYGFGLMD 450
    ||||||||||||||||||||||||||||||||||||||||||||||||||
401 ALEANPFLTWRDVQHVIVRTSRAGHLNANDWKTNAAGFKVSHLYGFGLMD 450
                     .         .         .         .         .
451 AEAMVMEAEKWTTVPRQHVCVESTDRQIKTIRPNSAVRSIYKASGCSDNP 500
    ||||||||||||||||||||||||||||||||||||||||||||||||||
451 AEAMVMEAEKWTTVPRQHVCVESTDRQIKTIRPNSAVRSIYKASGCSDNP 500
```

FIG. 12B

```
              .         .         .         .         .
501 NRHVNYLEHVVVRITITHPRRGDLAIYLTSPSGTRSQLLANRLFDHSMEG 550
    ||||||||||||||||||||||||||||||||||||||||||||||||||
501 NRHVNYLEHVVVRITITHPRRGDLAIYLTSPSGTRSQLLANRLFDHSMEG 550

              .         .         .         .         .
551 FKNWEFMTIHCWGERAAGDWVLEVYDTPSQLRNFKTPGKLKEWSLVLYGT 600
    ||||||||||||||||||||||||||||||||||||||||||||||||||
551 FKNWEFMTIHCWGERAAGDWVLEVYDTPSQLRNFKTPGKLKEWSLVLYGT 600

              .         .         .         .         .
601 SVRPYSPTNEFPKVERFRYSRVEDPTDDYGTEDYAGPCDPECSEVGCDGP 650
    ||.|||||||||||||||||||||||||||||||||||||||||||||||
601 SVQPYSPTNEFPKVERFRYSRVEDPTDDYGTEDYAGPCDPECSEVGCDGP 650

              .         .         .         .         .
651 GPDHCNDCLHYYYKLKNNTRICVSSCPPGHYHADKKRCRKCAPNCESCFG 700
    ||||||||||||||||||||||||||||||||||||||||||||||||||
651 GPDHCNDCLHYYYKLKNNTRICVSSCPPGHYHADKKRCRKCAPNCESCFG 700

              .         .         .         .         .
701 SHGDQCMSCKYGYFLNEETNSCVTHCPDGSYQDTKKNLCRKCSENCKTCT 750
    ||||||||||||||||||||||||||||||||||||||||||||||||||
701 SHGDQCMSCKYGYFLNEETNSCVTHCPDGSYQDTKKNLCRKCSENCKTCT 750
```

FIG. 12C

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```
                .         .         .         .         .
751 EFHNCTECRDGLSLQGSRCSVSCEDGRYFNGQDCQPCHRFCATCAGAGAD 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
751 EFHNCTECRDGLSLQGSRCSVSCEDGRYFNGQDCQPCHRFCATCAGAGAD 800
                .         .         .         .         .
801 GCINCTEGYFMEDGRCVQSCSISYYFDHSSENGYKSCKKCDISCLTCNGP 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
801 GCINCTEGYFMEDGRCVQSCSISYYFDHSSENGYKSCKKCDISCLTCNGP 850
                .         .         .         .         .
851 GFKNCTSCPSGYLLDLGMCQMGAICKDATEESWAEGGFCMLVKKNNLCQR 900
    ||||||||||||||||||||||||||||||||||||||||||||||||||
851 GFKNCTSCPSGYLLDLGMCQMGAICKDATEESWAEGGFCMLVKKNNLCQR 900
                .
901 KVLQQLCCKTCTFQG. 915
    |||||||||||||||
901 KVLQQLCCKTCTFQG* 916
```

FIG. 12D

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(57) Abstract

A cDNA clone encoding the human prohormone convertase PC5 was isolated from human adrenal gland messenger RNA. The deduced protein sequene would encode a 915 amino acid prepro PC5 which shares a very high degree of homology with the previously cloned rat and mouse homologues. PC5 mRNA is detected in multiple human tissues, including the brain, adrenal and thyroid glands, heart, placenta, lung and testes. PC5 mRNA was undetectable in the liver and is present at lower levels in skeletal muscle, kidney, pancreas, small intestine and stomach. Co-transfection of human PC5 and human prorenin expression vectors in cultured GH_4Cl cells leads to secretion of active renin. The activation of human prorenin by PC5 is dependent on a pair of basic amino acids at positions 42 and 43 of the prorenin prosegment and occurs only in cells containing dense core secretory granules. Human PC5 was co-localized with renin by immunohistochemistry in the zona glomerulosa of the adrenal gland suggesting that it could participate in the activation of a local renin-angiotensin system in the human adrenal cortex. PC5 is overexpressed in atherosclerotic coronary blood vessels. Silencing PC5 expression with a specific antisense oligonucleotide efficiently inhibited the proliferation of smooth muscle cells in culture. Furthermore, the antisense inhibited carotid stenosis in a carotoid injury model. These results indicate that silencing PC5 applies to the prevention of restenosis. PCs could be targets of choice for treating any proliferative diseases involving their action on a given growth factor. Finally, the antisense oligonucleotide PC5 is to be used for silencing the activity of this enzyme towards HIV gp160, since both coexist in $CD4^+$ T lymphocytes and the viral glycoprotein is cleavable by PC5.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00535

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/64 C12N15/11 C12N15/57

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MERCURE C. ET AL.: "Prohormone convertase PC5 is a candidate processing enzyme for prorenin in the human adrenal cortex" HYPERTENSION, vol. 28, no. 4, October 1996, DALLAS, US, pages 840-846, XP002046402 see the whole document ---	1-5

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 November 1997	10.03.98

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/CA 97/00535

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MIRANDA L. ET AL.: "Isolation of the human PC6 gene encoding the putative host protease for HIV-1 gp160 processing in CD4+ T lymphocytes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, July 1996, WASHINGTON US, pages 7695-7700, XP002046403 cited in the application	1,3-6,8, 12
Y	see the whole document	11, 13-15,18

Y	WO 94 15945 A (TEXAS BIOTECHNOLOGY CORPORATION) 21 July 1994 see abstract see page 2, line 10 - line 22	11, 13-15,18

A	DECROLY E ET AL: "THE CONVERTASES FURIN AND PC1 CAN BOTH CLEAVE THE HUMAN IMMUNODEFICIENCY VIRUS (HIV)-1 ENVELOPE GLYCOPROTEIN GP160 INTO GP120 (HIV-I SU) AND GP41 (HIV-I TM)" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 16, 22 April 1994, pages 12240-12247, XP002023318	8,12-18

A	HALÈNE C ET AL: "SPECIFIC REGULATION OF GENE EXPRESSION BY ANTISENSE, SENSE AND ANTIGENE NUCLEIC ACIDS" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1049, 1 January 1990, pages 99-125, XP000570355	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/00535

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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2. ☒ Claims Nos.: 1 8 11 15
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The definition of an enzyme by the term PC5 is not sufficient to define the subject matter as it is a non structural definition. The search has been performed according to the definition of the subject matter as given by the Sequences Nos. 1 and 2 of the seq. listing.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/CA 97/00535

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6, 8, 11-15, part. 16-18

   A purified human pro-protein converting enzyme isolated from adrenals, its DNA and aminoacid sequences and its uses.

2. Claims: 7, 9, 10, part. 16-18

   An antisense oligonucleotide and its uses

INTERNATIONAL SEARCH REPORT

Intern   ıal Application No
PCT/CA 97/00535

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9415945 A	21-07-1994	AU 6080194 A	15-08-1994
		CA 2152903 A	21-07-1994
		EP 0677055 A	18-10-1995